

Right so that avian EG cells are obtained.

P2 3. (Amended) The method of Claim 2, wherein the maximal amounts of said growth factors range from about two times to one hundred times said minimal amounts.

P3 9. (Amended) The method of Claim 1, wherein the avian EG cells produce mouse-stage specific antigen 1, and/or react with EMA-1 or MC-480 monoclonal antibody.

P4 12. (Amended) The method of Claim 1, which further comprises:
(iii) transfecting or transforming the resultant EG cells with a nucleic acid sequence.

Sub C3 13. (Amended) The method of Claim 12, wherein said nucleic acid sequence encodes a polypeptide and is expressed in an egg.

14. (Thrice Amended) A method of producing chimeric avians comprising:
(i) isolating primordial germ cells (PGCs) from an avian;
(ii) culturing the PGCs in the absence of a feeder layer in a tissue culture medium containing at least the following growth factors;
(1) leukemia inhibitory factor (LIF),
(2) basic fibroblast growth factor (bFGF),
(3) stem cell factor (SCF) and
(4) insulin-like growth factor (IGF)
for a sufficient time to produce embryonic germ (EG) cells;

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(iii) transferring said EG cells into a recipient avian embryo; and
(iv) obtaining a germline and somatic cell chimeric avian.

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18. (Amended) The method of Claim 17, wherein said nucleic acid sequence encodes a polypeptide and is expressed in an egg of an avian embryo having said nucleic acid sequence.

19. (Amended) The method of Claim 17, wherein said nucleic acid encodes a polypeptide that can be recovered from the systemic circulatory system, body fluids, or tissues of an avian having said nucleic acid sequence.

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21. (Amended) The method of Claim 17, wherein said nucleic acid encodes a polypeptide that is a growth factor or an enzyme.

22. (Amended) The method of Claim 12, wherein said nucleic acid encodes a polypeptide that can be recovered from the systemic circulatory system, body fluids, or tissues of an avian having said nucleic acid sequence.

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23. (Amended) The method of Claim 1, wherein said nucleic acid encodes a polypeptide that is a growth factor or an enzyme.

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25. (Amended) A method of producing germline chimeric avians comprising:
(i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

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(ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF);

(iii) transferring said PGCs into a Stage XII-XIV recipient avian embryo; and

(iv) obtaining germline chimeric avians having germline cells that have the genotype of said PGCs.

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26. (Amended) A method of producing germline or somatic cell chimeric avians which comprises:

(i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF),

for a sufficient time to produce embryonic germ (EG) cells;

(iii) transferring said EGs into a recipient Stage X avian embryo of the same species as the avian used to obtain said isolated PGCs;

(iv) allowing said recipient avian to develop into a germline or somatic cell chimeric avian having germline and somatic cells that have the genotype of said PGCs.

Jul 6 '03 > 27. (Amended) A method for producing avian embryonic germ (EG) cells

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comprising:

(i) isolating a population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) culturing said population of PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:

(1) leukemia inhibitory factor (LIF),
(2) basic fibroblast growth factor (bFGF),
(3) stem cell factor (SCF) and
(4) insulin-like growth factor (IGF)

so that avian EG cells are produced.

28. (Amended) A method for producing a chimeric avian comprising:

(i) isolating a population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) culturing said population of PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:

(1) leukemia inhibitory factor (LIF),
(2) basic fibroblast growth factor (bFGF),
(3) stem cell factor (SCF) and

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(4) insulin-like growth factor (IGF);

(iii) transferring said PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs;

(iv) allowing said recipient avian embryo to develop into a chimeric avian.

29. (Amended) A method for producing a germline chimeric avian comprising:

(i) isolating a population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) culturing said population of PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:

(1) leukemia inhibitory factor (LIF),

(2) basic fibroblast growth factor (bFGF),

(3) stem cell factor (SCF) and

(4) insulin-like growth factor (IGF);

(iii) transferring said purified PGCs into a recipient Stage XII-XIV avian embryo of the same species as the avian used to obtain said isolated, purified PGCs; and

(iv) allowing said recipient avian embryo to develop into a germline chimeric avian.

30. (Amended) A method for producing germline or somatic cell chimeric avians comprising:

(i) isolating a population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) culturing said population of PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF);

(iii) transferring said EGs into a recipient Stage X avian embryo of the same species as the avian used to obtain said isolated PGCs; and

(iv) allowing said recipient avian embryo to develop into a germline or somatic cell chimeric avian.
